The ATP-Sensitive K^+ Channel *ABCC8* S1369A Type 2 Diabetes Risk Variant Increases MgATPase Activity

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Pancreatic β-cell ATP-sensitive K^+ (K_{ATP}) channels are composed of Kir6.2 and SUR1 subunits encoded by the *KCNJ11* and *ABCC8* genes, respectively. Although rare monogenic activating mutations in these genes cause overt neonatal diabetes, the common variants E23K (*KCNJ11*) and S1369A (*ABCC8*) form a tightly heritable haplotype that is associated with an increased susceptibility to type 2 diabetes (T2D) risk. However, the molecular mechanism(s) underlying this risk remain to be elucidated. A homology model of the SUR1 nucleotide-binding domains (NBDs) indicates that residue 1369 is in close proximity to the major MgATPase site. Therefore, we investigated the intrinsic MgATPase activity of K_{ATP} channels containing these variants. Electrophysiological and biochemical techniques were used to study the MgATPase activity of recombinant human K_{ATP} channels or glutathione S-transferase and NBD2 fusion proteins containing the E23/S1369 (nonrisk) or K23/A1369 (risk) variant haplotypes. K_{ATP} channels containing the K23/A1369 haplotype displayed a significantly increased stimulation by guanosine triphosphate compared with the E23/S1369 haplotype (3.2- vs. 1.8-fold). This effect was dependent on the presence of the A1369 variant and was lost in the absence of Mg^{2+} ions or in the presence of the MgATPase inhibitor beryllium fluoride. Direct biochemical assays also confirmed an increase in MgATPase activity in NBD2 fusion proteins containing the A1369 variant. Our findings demonstrate that the A1369 variant increases K_{ATP} channel MgATPase activity, providing a plausible molecular mechanism by which the K23/A1369 haplotype increases susceptibility to T2D in humans homozygous for these variants.
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In pancreatic β-cells, ATP-sensitive K^+ (K_{ATP}) channels play a vital role by acting as intracellular sensors of glucose metabolism, and thus the control of insulin secretion (1,2). ATP inhibits K_{ATP} channels, but ADP stimulates channel activity by antagonizing the inhibitory action of ATP. It is now accepted that the ATP/ADP ratio within the cell is the major determinant of channel activity (1–4), and thus insulin secretion. Glucose-mediated K_{ATP} channel closure is a key triggering event that initiates first-phase (K_{ATP} channel dependent) insulin secretion, and increased K_{ATP} channel activity may contribute to the blunted first-phase secretion observed in type 2 diabetes (T2D) (5–7).

In the pancreatic β-cell and central nervous system, the K_{ATP} channel is a hetero-octamer composed of the SUR1 and Kir6.2 subunits encoded by the *ABCC8* and *KCNJ11* genes, respectively (8). SUR1 and Kir6.2 monogenic activating mutations have been found to underlie mild to severe forms of neonatal diabetes and developmental delay (9–12). Thus, altered genetic makeup of K_{ATP} channels can cause insulin secretory disorders of varying severity.

However, common genetic variants with more subtle effects on K_{ATP} channel activity may display no overt clinical phenotype yet increase T2D susceptibility. In this regard, candidate gene and genome-wide association studies have identified the E23K single nucleotide variant (K23, rs5219) within the *KCNJ11* Kir6.2 K_{ATP} channel subunit gene as being associated with T2D and impaired glucose-stimulated insulin secretion in ~20% of Caucasians with T2D (13–16). Moreover, the K23 variant has also been shown to be associated with T2D in almost all ethnic groups tested to date (17–20). Despite intense research on the K23 variant, studies on the molecular mechanism(s) that predispose to T2D have produced conflicting data (21–26), suggesting perhaps that a more complex process underlies the association of K23 with T2D.

It is important to note that the K23 variant is tightly associated with the SUR1 S1369A *ABCC8* variant (A1369 rs757110) forming a haplotype, such that >95% of people with two copies of K23 also possess two copies of A1369 (27). Moreover, the *ABCC8* and *KCNJ11* genes are located adjacent to each other at the same chromosomal position (11p15.1), further supporting a tight inheritable haplotype. In this regard, the very high association between the A1369 *ABCC8* and the K23 *KCNJ11* variants (27) confirms that it is this haplotype that represents the variant composition associated with an increased risk for T2D and indicates that there may be a distinct K_{ATP} channel phenotype associated with the homozygous K23/A1369 T2D risk haplotype compared with the more prevalent E23/S1369 nonrisk haplotype or E23 alone. Although our recent data have provided some initial insights into the properties of the K23/A1369 T2D risk haplotype (26), the precise molecular processes that underlie an increased risk for T2D remain to be elucidated.

The SUR1 subunit possesses two NBDs (NBD1 and 2) that dimerize to form the MgADP sensor and the catalytic site of the channel's intrinsic MgATPase activity (2,28–30) (Fig. 1). MgATP can inhibit the channel via interactions with the Kir6.2 subunit, whereas MgADP antagonizes this inhibition by interactions with the NBD1/NBD2 dimers in SUR1. MgATP also serves as a substrate for the intrinsic MgATPase activity of the channel complex, generating MgADP (28). Therefore, the overall channel activity is determined by a dynamic balance between MgATP and MgADP levels that are regulated locally by the MgATPase activity of the channel and by alterations in cellular metabolism. The importance of MgATPase activity is highlighted by the fact that several rare monogenic mutations in NBD2 (R1380L/C) (Fig. 1) lead to increased MgATPase activity that results in greatly reduced inhibition by ATP, enhanced K_{ATP} channel activity, and overt neonatal diabetes

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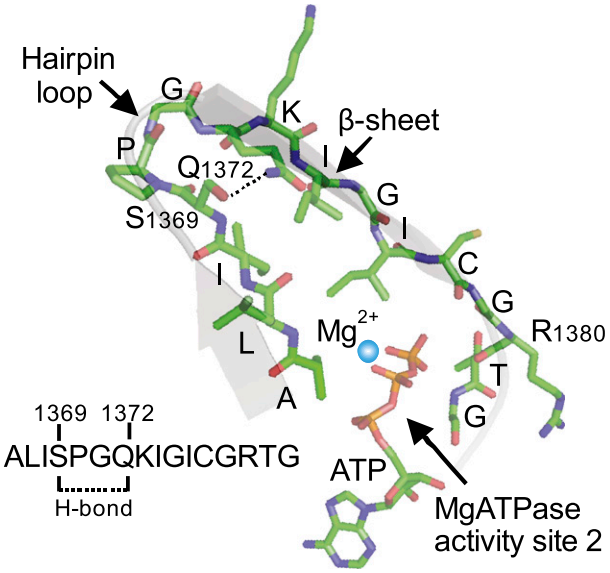

FIG. 1. In silico analysis of the hairpin loop/β-sheet region proximal to the MgATPase activity site 2 in NBD2. The hydroxyl group on the side chain of residue S1369 is predicted to form a hydrogen bond with the NH_2-terminal group of the side chain on Q1372. Dotted line denotes the hydrogen bond. Residue R1380 is highlighted where mutations are known to cause monogenic neonatal diabetes via increases in MgATPase activity (29). Analysis was performed using PyMOL software on the SUR1 NBD1/2 dimer homology model of the prokaryotic ABC protein MJ0796 constructed by Masia and Nichols (30).

in humans, because insulin secretion is severely reduced (29,31). In the current study, we used an in silico homology model of the NBD1 and 2 dimer (30) that predicts the A1369 variant is adjacent to the site of catalytic MgATPase activity in NBD2 and may subtly alter the secondary or tertiary structure of the NBD1/NBD2 dimer, leading to increases in MgATPase activity. To test this notion, we performed patch-clamp and biochemical MgATPase assays on recombinant human K_{ATP} channels or glutathione S-transferase (GST)-NBD2 fusion proteins containing the nonrisk E23/S1369 or the T2D risk K23/A1369 haplotype.

RESEARCH DESIGN AND METHODS

Cell culture, transfection, and electrophysiology. Cultured tsA201 cells were transfected with the *KCNJ11* and *ABCC8* clones using the calcium phosphate precipitation technique (32). Transfected cells were identified using fluorescent optics in combination with coexpression of a green fluorescent protein plasmid (Life Technologies, Gaithersburg, MD). Macroscopic K_{ATP} channel recordings were then performed 48–72 h after transfection. The excised inside-out patch-clamp technique was used to measure macroscopic recombinant K_{ATP} channel currents in transfected tsA201 cells as described in detail previously (32). Experiments were performed at room temperature. The bath solution for recording in the absence of Mg^{2+} ions contained no $MgCl_2$, and 2 mmol/L EGTA were added. Beryllium fluoride (BeF_2) was prepared as a 33% stock solution dissolved in 50 mmol/L KF to produce the γ-phosphate analogs BeF_X (BeF_3^- and BeF_4^{2-}) (29). For preparing bath solutions to be used for experiments in the presence of BeFx, 50 mmol/L KCl were replaced with 50 mmol/L KF.

Experimental compounds. BeF_2, MgATP, and Na^+ salts of guanosine triphosphate (GTP) and guanosine diphosphate (GDP) were obtained from Sigma-Aldrich (Oakville, ON, Canada). ATP, GTP, and GDP were prepared as 100 mmol/L stocks in double deionized H_2O immediately before use.

Molecular biology. The human K_{ATP} channel Kir6.2 and SUR1 subunit clones were provided by Dr. J. Bryan (Pacific Northwest Diabetes Research Institute, Seattle, WA). The E23K/S1369A variants and the Q1372A mutation were introduced into the *KCNJ11* and *ABCC8* cDNAs, respectively, using site-directed mutagenesis (QuikChange; Stratagene, La Jolla, CA) and confirmed by sequence analysis.

In silico homology model. The homology model of the SUR1 NBD1 and NBD2 dimer (30), based on the prokaryotic ATP binding cassette protein crystal structure of MJ0796 (Protein Data Bank accession number 1F30) was provided by Dr. C.G. Nichols (Washington University, St. Louis, MO) and used to predict the location of key regions and residues in the NBD1/2 dimer using PyMOL software (Fig. 1).

Direct MgATPase assays. A 681 base pair–long fragment of NBD2 (aa 1301–1528), containing the S1369 or A1369 variant, was subcloned into the pGEX-4T-1 expression vector. The recombinant plasmids were sequenced and then transformed into BL21 (DE3) cells for protein expression. The GST-NBD2 fusion proteins were detected with a monoclonal anti–GST tag antibody (1:5,000 dilution, Santa Cruz Biotechnology, Inc., Santa Cruz, CA). After purification, proteins (50 μg) were incubated at 40°C for 30 min followed by 1-h incubation at 4°C to allow homogeneous dimerization of the NBD2 monomers. MgATPase activities of NBD2 dimers were determined at 37°C by monitoring the rate of production of the fluorophore resorufin, which was coupled stoichiometrically to the formation of ADP (ADP Quest; DiscoverX, Fremont, CA). ADP-free ATP (ATP-Gold, DiscoverX) was used as substrate. Resorufin formation was measured continuously in a fluorescence microplate reader (Gemini XPS; Molecular Devices, Sunnyvale, CA) with excitation and emission wavelengths of 560 and 591 nm, respectively, and with an emission filter at 590 nm. Initial rates (v) were determined in quadruplicate at several ATP concentrations from the slopes of early pseudo-linear portions of fluorescence (relative fluorescence unit) versus time curves (SoftMax Pro software; Molecular Devices). Velocity data were fitted with the Michaelis-Menten equation to obtain K_M and V_{max} values.

Statistical analysis. Macroscopic K_{ATP} channel currents were normalized and expressed as changes in test current relative to control current. Macroscopic current analysis was performed using pClamp 10.0 (Axon Instruments, Foster City, CA) and Origin 6.0 software. Statistical significance was assessed using the unpaired Student t test or one-way ANOVA with a Bonferroni post hoc test. $P < 0.05$ was considered statistically significant. Data are expressed as the mean ± SEM.

RESULTS

Our recent data indicated that K_{ATP} channels containing the *ABCC8* A1369 variant are less sensitive to inhibition by MgATP, whereas the intrinsic open probability at zero ATP remained unchanged (26). There are three possible explanations for these observations indicated as follows: *1*) K_{ATP} channels containing the A1369 variant are more sensitive to activation by MgADP; *2*) the Kir6.2-mediated ATP inhibition is reduced; or *3*) the enzymatic MgATPase activity of the channel is increased, leading to elevated local levels of MgADP that relieve ATP inhibition. The first two possibilities are unlikely because we previously demonstrated that the MgADP stimulatory effects are unaltered in the K23/A1369 channel haplotype, and the presence of the *KCNJ11* (Kir6.2) E23 or K23 variants do not change the IC_{50} for ATP inhibition via the Kir6.2 subunit (26). Therefore, we tested the notion that the observed decrease in MgATP sensitivity in K_{ATP} channels containing the *ABCC8* A1369 variant is caused by an increase in the intrinsic MgATPase activity of the K_{ATP} channel complex.

Analysis of the NBD1/2 dimer homology model (30) places residue 1369 within a β-sheet/hairpin loop that is ~10 residues proximal to the primary MgATPase activity site 2 in the dimer (Fig. 1). Because MgATP can activate the K_{ATP} channel complex after being converted to MgADP through intrinsic MgATPase activity, we explored whether the presence of the A1369 variant affects K_{ATP} channel activity by altering the MgATPase enzymatic activity of SUR1. It is well established that the nucleotide GTP can be used as a substrate for SUR1 ATPase activity but is a very weak inhibitor of channel activity via the Kir6.2 subunit (32,33). However, GDP (similar to ADP) generated by SUR1 MgATPase activity has a stimulatory effect on K_{ATP} channels. By monitoring changes in K_{ATP} channel activity, GTP can be used to quantify MgATPase activity.

To evaluate the effects of the K23 and A1369 T2D risk variants on MgATPase activity, we measured GTP-elicited current responses in recombinant human K_{ATP} channels containing the E23/S1369 nonrisk haplotype or the K23/A1369 T2D risk haplotype using the conventional excised inside-out patch technique. In the presence of 0.1 mmol/L ATP, GTP (1 mmol/L) stimulated K_{ATP} channel activity to a significantly greater extent in channels containing the K23/A1369 T2D risk haplotype compared with channels containing the nonrisk E23/S1369 haplotype (3.24 ± 0.05- vs. 1.83 ± 0.14-fold increase, respectively, $P < 0.0001$) (Fig. 2*A*, *B*, and *E*). To test whether this observed enhancement of GTP stimulation was due to the presence of either the K23 or A1369 variant alone, we expressed quasi-heterologous K_{ATP} channels containing E23 and A1369 and observed that GTP caused a similar enhancement of channel activity to the K23/A1369 T2D risk variant (2.99 ± 0.14-fold for E23/A1369 vs. 1.83 ± 0.14-fold increase for E23/S1369, $P <$ 0.0001) (Fig. *2C* and *E*). These results indicate that it is the A1369 variant (and not K23) that is responsible for the enhanced GTP stimulatory effect, suggesting that the K_{ATP} channel complex's intrinsic MgATPase activity is enhanced by the presence of the A1369 variant (Fig. 2*F*). Homology modeling of NBD2 predicts that the S1369 residue forms a hydrogen bond with Q1372 and that the A1369 variant lacks the ability to form this bond (Fig. 1). Accordingly, we introduced the Q1372A mutation in the presence of S1369 (E23/S1369-Q1372A) to test whether the GTP stimulatory effect is similarly enhanced. The Q1372A mutation significantly enhanced the stimulatory effect of GTP compared with K_{ATP} channels containing E23/S1369 and Q1372 (2.42 ± 0.15- vs. 1.83 ± 0.14-fold, $P < 0.0001$) (Fig. 2*D* and *E*).

To provide further evidence for the A1369 variant altering MgATPase activity, we performed similar experiments in the absence of Mg^{2+} ions or in the presence of the MgATPase inhibitor BeF_2. Mg^{2+} ions are a necessary cofactor required for MgATPase activity, and removal of Mg^{2+} ions from the intracellular solution should prevent GTP-mediated stimulation of K_{ATP} channels if MgATPase activity is responsible. In the absence of Mg^{2+} ions, no GTP stimulatory effect was observed in K_{ATP} channels containing the E23/S1369 nonrisk or K23/A1369 T2D risk haplotype (1.06 ± 0.01- vs. 1.06 ± 0.02-fold increase, respectively) (Fig. 3). In the absence of Mg^{2+} ions, the IC_{50} for ATP inhibition was not significantly different for the E23/S1369 nonrisk or K23/A1369 T2D risk haplotype (7.7 ± 2.2 vs. 7.9 ± 1.3 μmol/L) (Fig. 3*D*).

BeF_2 (BeF_3^- and BeF_4^{2-}, abbreviated in text as BeF_X) is a potent inhibitor of many ATPases, including K_{ATP} channels, locking the ATPase in a prehydrolytic state by stable trapping of the MgADP-BeF_X complex in the catalytic sites (28,34–37). We observed that the stimulatory effect of GTP (1 mmol/L) is lost in the presence of 1 mmol/L BeF_X in K_{ATP} channels containing the E23/S1369 or K23/A1369 haplotype (Fig. 4*A*–*C*). If the observed GTP stimulatory

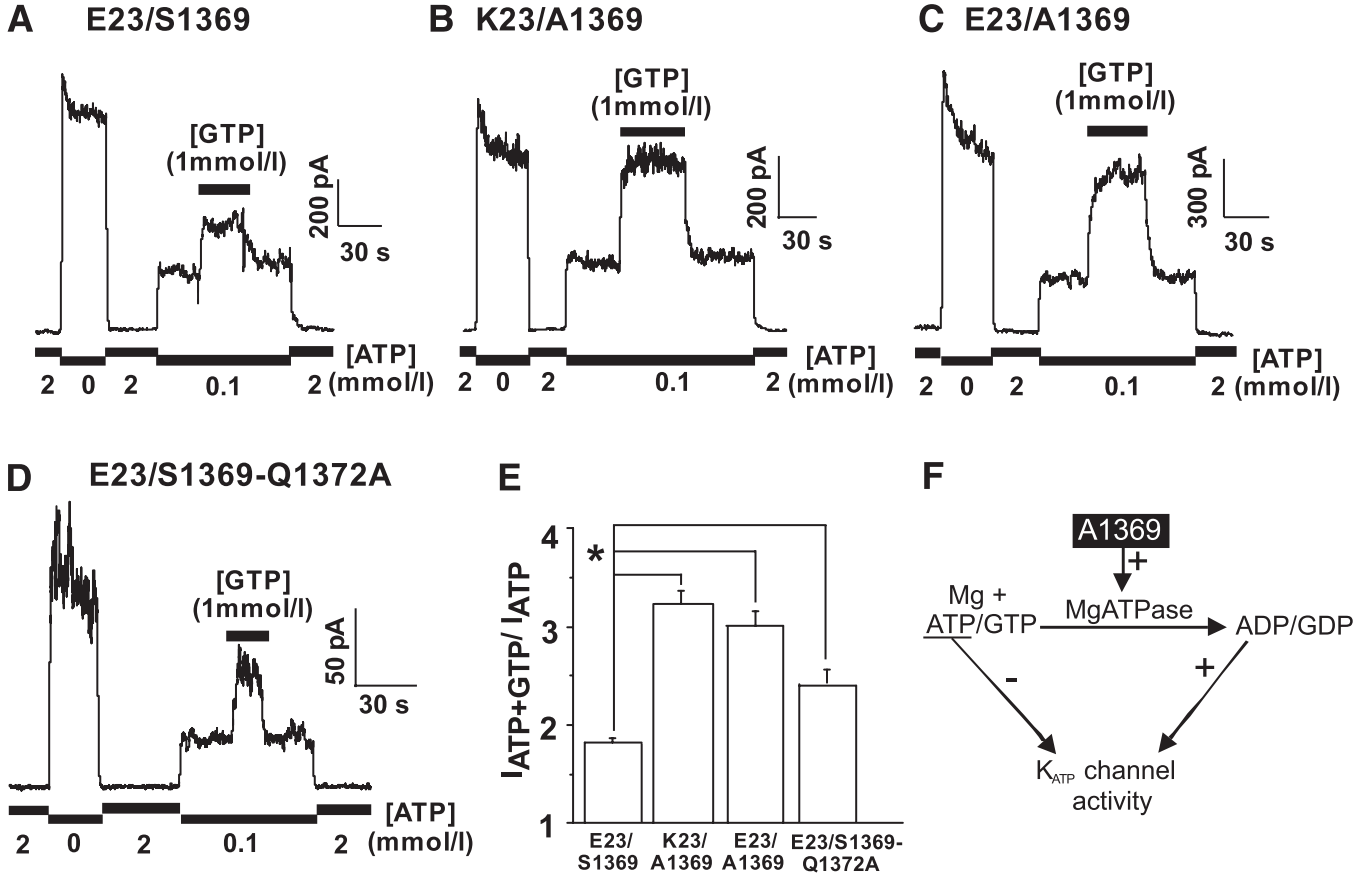

FIG. 2. GTP has greater stimulatory effect on K_{ATP} channels containing the *ABCC8* A1369 variant compared with the S1369 variant (*F*). *A–D*: Representative macroscopic currents of recombinant human K_{ATP} channels at different MgATP concentrations and before/after exposure to 1 mmol/L GTP. *E*: Grouped data showing a significant increase in the stimulatory effect of GTP in channels containing the A1369 variant and in channels containing the E23/S1369 variants with the Q1372A mutation (n = 10–20 patches per group). *$P < 0.0001$.

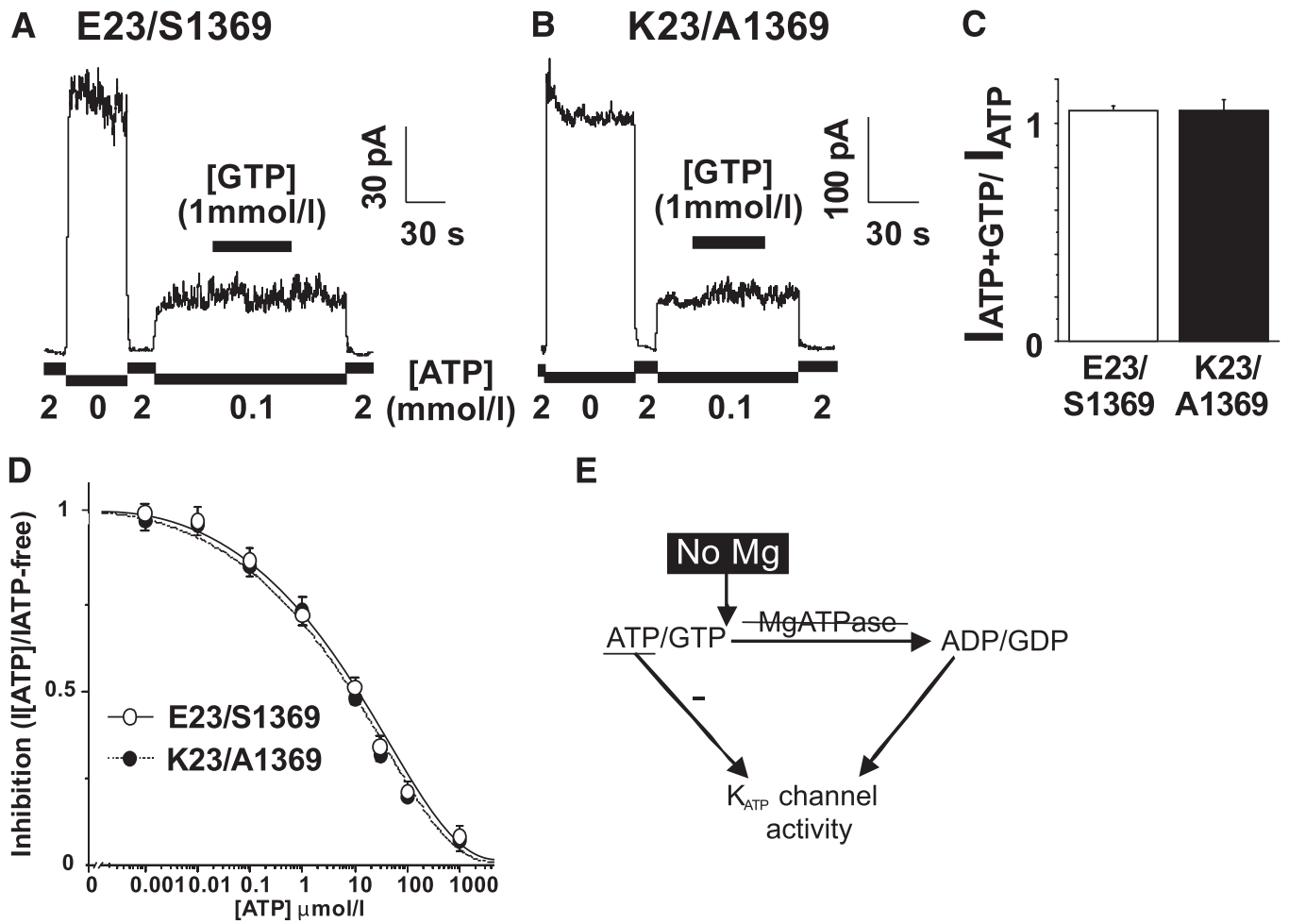

FIG. 3. In the absence of Mg^{2+} ions, GTP has no stimulatory effect on K_{ATP} channels containing the E23/S1369 or K23/A1369 variants (*E*). *A* and *B*: Representative macroscopic current recordings from the excised inside-out patches of tsA201 cells membranes expressing recombinant human K_{ATP} channels containing the K23/A1369 or E23/S1369 haplotypes, respectively. *C*: Grouped data from 6–8 patches per group. No significant stimulation of current by GTP was observed. *D*: Mg^{2+}-free ATP-inhibition curves for K_{ATP} channels containing the E23/S1369 or K23/A1369 variants (n = 4–11 patches per ATP concentration).

effect is due to GTP hydrolysis via MgATPase activity, then this effect should be lost with the nonhydrolyzable GTP analog GMP-PNP. Indeed, GMP-PNP did not enhance currents recorded from K_{ATP} channels containing the E23/S1369 or K23/A1369 haplotype (Fig. 5). These results provide further evidence for the observed stimulatory effects of GTP being mediated by the intrinsic MgATPase activity of the K_{ATP} channel complex.

Although we previously demonstrated that the ADP sensitivity is similar in K_{ATP} channels containing the E23/S1369 or K23/A1369 haplotype (26), the possibility remains that the GDP-mediated stimulation may be different in these two K_{ATP} channel haplotypes. Accordingly, we measured the current responses of the two K_{ATP} channel haplotypes to 0.1 or 1 mmol/L GDP while the channels were partially inhibited by 0.1 mmol/L ATP. We observed no significant difference in the ability of GDP to stimulate K_{ATP} channels containing the E23/S1369 or K23/A1369 haplotype, indicating that both variants are equally sensitive to GDP (Fig. 6) (1.63 ± 0.05 vs. 1.79 ± 0.06 at 0.1 mmol/L GDP and 3.37 ± 0.2 vs. 3.13 ± 0.15 at 1 mmol/L GDP for the E23/S1369 and K23/A1369 haplotypes, respectively). These results suggest that it is not GDP sensitivity that is altered in the K23/A1369 haplotype but rather that the elevated generation of GDP from increased MgATPase activity is responsible for the observed differences in the GTP stimulatory effect (Fig. 2).

Candidate gene and genome-wide association studies indicate that T2D risk is only associated with the homozygous state, in other words, K_{ATP} channels containing only the K23 and A1369 variants. Therefore, we sought to determine whether K_{ATP} channel subunits containing a mixture of all the E23, K23, S1369, and A1369 variants would display an enhanced GTP stimulatory effect. To achieve this, we transfected tsA201 cells with equimolar amounts of the individual plasmids containing all four variants to generate the expression of quasi-heterozygous K_{ATP} channels. No significant difference was observed between the stimulatory effects of GTP (1 mmol/L) in K_{ATP} channels containing the E23/S1369 nonrisk haplotype and K_{ATP} channels composed of subunits containing the E23, K23, S169, and A1369 variants (E+K/S+A) (Fig. 7*A*–*C*).

The electrophysiological data presented above provide good evidence for enhanced MgATPase activity in K_{ATP} channels containing the A1369 variant. However, we sought further confirmation of this concept using a direct assay of MgATPase activity in GST-NBD2 fusion proteins containing the S1369 or A1369 variants. A significant increase in MgATP binding and MgATPase activity was observed in

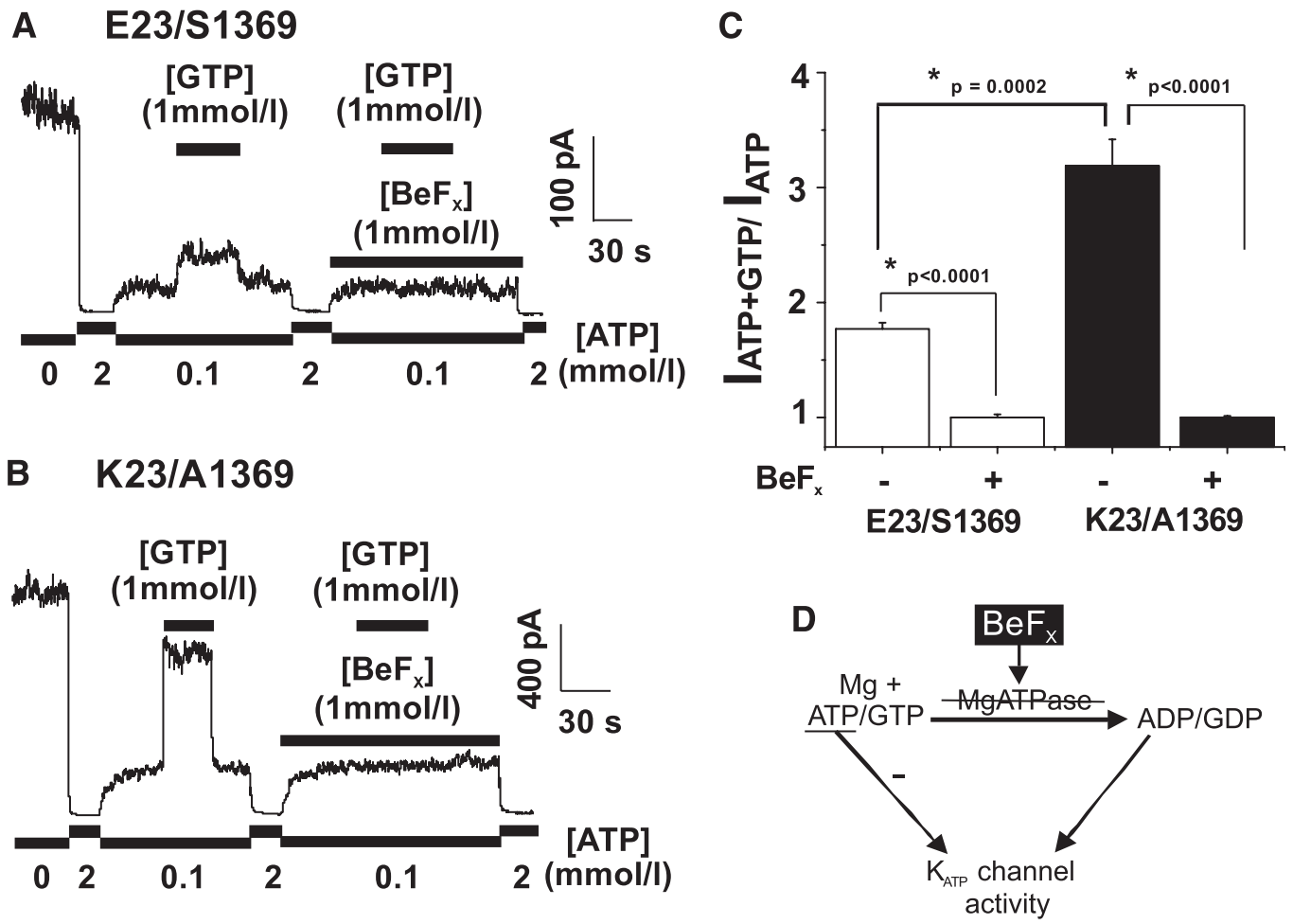

FIG. 4. In the presence of the ATPase inhibitor BeF_X (1 mmol/L), the GTP stimulatory effect is removed (*D*). *A* and *B*: Representative macroscopic current recordings of recombinant human K_{ATP} channels containing the E23/S1369 or K23/A1369 haplotypes, respectively. *C*: Grouped data (n = 9–10 patches per group) demonstrating a significant difference between the stimulatory effects of GTP in the absence or presence of BeF_X.

NBD2 containing the A1369 variant compared with S1369 (K_M = 82 ± 1.7 vs. 130 ± 16 μmol/L; V_{max} = 694 ± 37 vs. 365 ± 16 pmol ADP/min/mg, $P < 0.05$, n = 3) for A1369 and S1369, respectively (Fig. 7*D*). To provide further evidence that ATP hydrolysis is required, ATP was replaced with the nonhydrolyzable analog 5′-adenylylimidodiphosphate (AMP-PNP) in MgATPase assays using the S1369 GST-NBD2 fusion protein. As expected, the MgATPase activity of the GST-NBD2 fusion protein was abolished with the replacement of ATP with AMP-PNP (Fig. 7*E*). The ATP inhibitor BeF_x used in this study inhibited the MgATPase activity of the GST-NBD2 S1369 fusion protein with an IC_{50} of 40 μmol/L (data not shown).

DISCUSSION

Previous studies investigating the precise molecular mechanism(s) that underpin the documented association of the K_{ATP} channel *KCNJ11* K23 variant have yielded variable results (21–25). This may reflect that in these earlier studies, the K23 variant was investigated in recombinant K_{ATP} channels composed of subunits from several different species without controlling for A1369. Our recent work was the first to document the properties and pharmacology of human K_{ATP} channels that contain the K23 and A1369 T2D risk haplotype and indicated that it is the A1369 variant, but not K23, that alters the MgATP-sensing properties of the K_{ATP} channel (26). The underlying molecular mechanism for the reduced MgATP inhibition observed in human K_{ATP} channels containing the common K23/A1369 T2D risk haplotype is of obvious importance to understand the development of T2D in humans carrying two copies of this haplotype. Insights into this molecular mechanism can be gleaned from previous studies on rare heterozygous *ABCC8* mutations (R1380L and R1380C) that cause neonatal diabetes via increases in MgATPase activity (29). Homology modeling places R1380 within the predicted major MgATPase catalytic site 2 (Fig. 1), increasing MgATPase activity and rendering the K_{ATP} channel markedly less sensitive to MgATP inhibition compared with free ATP inhibition, leading to severely impaired insulin secretion in affected patients (29). Results from the current study now demonstrate that the A1369 variant also increases MgATPase activity as measured in intact K_{ATP} channels using electrophysiological techniques (Figs. 2–5) and in a direct assay of NBD2 MgATPase activity (Fig. 7*D* and *E*). Although the underlying mechanism of increased MgATPase activity may be the same for rare monogenic mutations at residue 1380 and for the A1369 variant (as demonstrated in this study), the clinical phenotype is strikingly different: overt neonatal diabetes versus T2D risk. This may be explained by the difference in the magnitude of channel

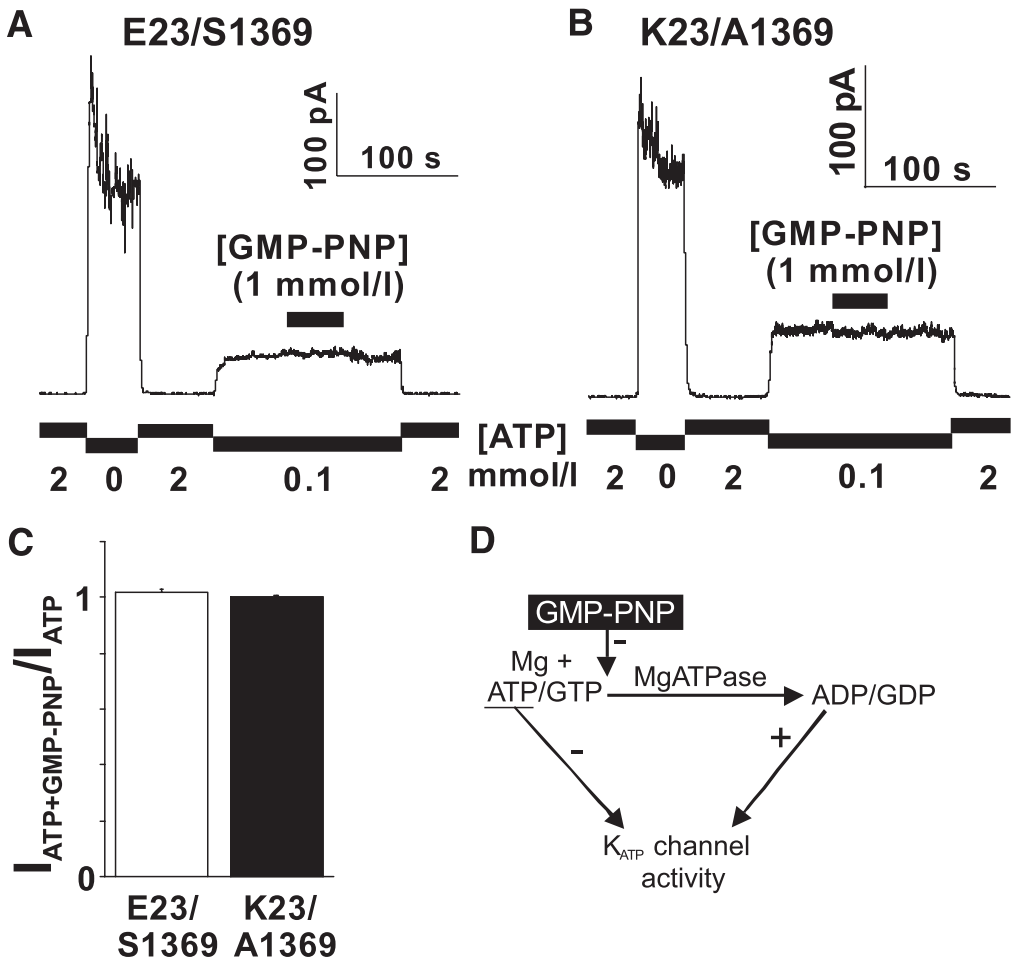

FIG. 5. The nonhydrolyzable GTP analog GMP-PNP does not stimulate K_{ATP} channel activity (*D*). *A* and *B*: Representative macroscopic current recordings of recombinant human K_{ATP} channels containing the E23/S1369 or K23/A1369 haplotypes in response to 1 mmol/L GMP-PNP. *C*: Grouped data demonstrating no significant difference between currents in the absence or presence of 1 mmol/L GMP-PNP (n = 11–12 patches per group).

activation at physiological millimolar ATP concentrations. Monogenic mutations at residue 1380 result in large increases in K_{ATP} channel activity at millimolar MgATP (29), effectively clamping β-cells in an electrically quiescent state preventing insulin release. Indeed, inhibition of overactive K_{ATP} channels with sulfonylurea therapy has been shown to be an effective therapy in many cases of neonatal diabetes resulting from K_{ATP} channel activation mutations (38). In contrast, the A1369 variant may subtly increase the K_{ATP} channel's intrinsic MgATPase activity, leading only to modest increases in K_{ATP} channel activity at millimolar ATP that would oppose, but not prevent, insulin secretion and thus increase the risk for T2D without precipitating overt neonatal diabetes. This latter concept is supported by our previous observation that the MgATP sensitivity of K_{ATP} channels containing the A1369 variant is slightly reduced, leading only to small but measurable increases in single K_{ATP} channel activity at millimolar ATP (26). From a molecular perspective, analysis of the SUR1 NBD1/2 dimer homology model (30) places the 1369 residue adjacent to a hairpin loop in the β-sheet that forms the backbone of the NBD2 portion of the major MgATPase catalytic site 2 (Fig. 1). Therefore, substitution of Ser to an Ala at residue 1369 may alter the secondary or tertiary structure of this region, resulting in an enhanced MgATPase activity of the NBD1/2 dimer. Indeed, our results provide strong evidence for this concept because the increased GTP stimulatory effect in channels containing the A1369 variant (Fig. 2) is not due to altered GDP sensitivity (Fig. 5) but is lost in the absence of Mg^{2+} ions in the presence of the ATPase inhibitor BeF_X or with the nonhydrolyzable GTP analog GMP-PNP (Figs. 3–5). Furthermore, an assay of MgATPase activity of GST-NBD2 fusion proteins containing the S1369 or A1369 variant also confirmed that the A1369 directly increases MgATPase activity (Fig. 7*D* and *E*). It should be noted that a previous study reported increased GDP sensitivity in K_{ATP} channels containing the K23 variant, although K_{ATP} channel subunit clones from several species were used, and the importance of the A1369 variant was unknown at the time and thus not controlled for (23).

Although it is not possible to determine conclusively the exact structural consequences of the S1369A substitution from analysis of homology models alone, it is nevertheless possible to speculate on the possible mechanism(s). The side chain of Ser at residue 1369 is modeled to point toward the hairpin loop's interior with the terminal hydroxyl group hydrogen bonding with the NH_2-terminal group on the side chain of Q1372 (Fig. 1). This hydrogen bond would likely constrain the structure of the hairpin loop and the β-sheet proximal to the major MgATPase activity site 2

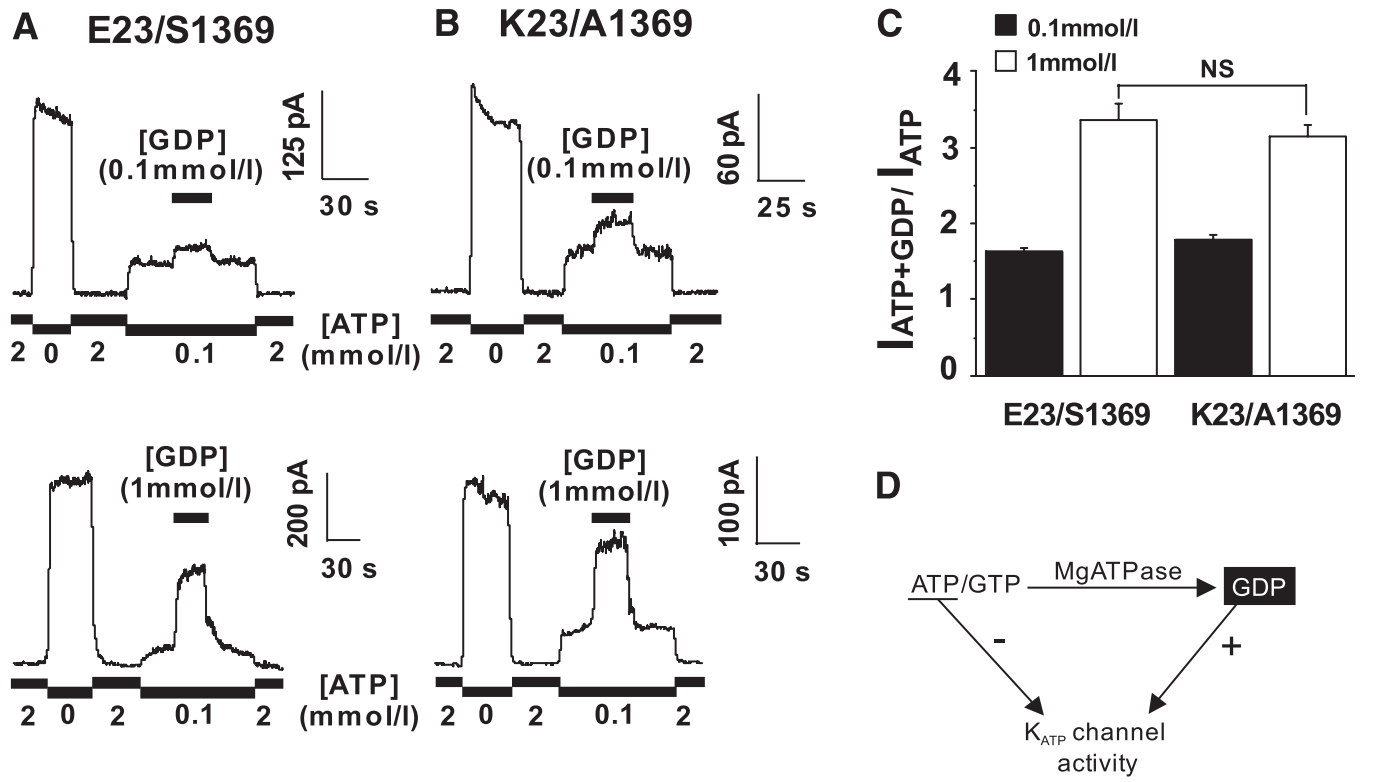

FIG. 6. GDP has a similar stimulatory effect on K_{ATP} channels containing the E23/S1369 or K23/A1369 haplotypes (*D*). *A* and *B*: Representative macroscopic current recordings of recombinant human K_{ATP} channel activity in response to GDP (0.1 or 1 mmol/L). *C*: Grouped data (n = 9–13 patches per group) demonstrating no significant differences in the magnitude of current stimulation by GDP. NS, not significant.

(Fig. 1). Substitution of the Ser with an Ala at position 1369 (A1369) removes only the side-chain terminal hydroxyl group that would prevent any hydrogen bond formation with Q1372. This would effectively reduce the structural constraint on the hairpin loop, allowing a greater movement in this region resulting in the observed increase in MgATPase activity. This concept is supported further by our data showing that introduction of the Q1372A mutation into channels containing the S1369 variant enhanced the GTP stimulatory effect (Fig. 2*D* and *E*). We therefore propose that the A1369 T2D risk variant causes subtle changes in the conformation of the dimer structure proximal to the NBD2 region that forms part of the MgATPase catalytic site 2.

The results obtained in this study further confirm that it is the A1369 variant and not K23 that alters the observed GTP stimulatory effect via increased MgATPase activity because the enhanced GTP stimulatory effect was similar in K_{ATP} channels containing E23 or K23 (Fig. 2*C* and *E*). These results are consistent with our previous findings indicating that the A1369 variant, and not the K23 variant, is responsible for the observed reduction in MgATP sensitivity (26). Our results further highlight that future mechanistic studies on the K23 variant should include the A1369 variant (27).

Previous candidate gene and genome-wide association studies have shown that the association of the *KCNJ11* K23 and *ABCC8* A1369 variants with T2D is present only in the homozygous state (37,39). These clinical findings suggest that all eight subunits in the K_{ATP} channel hetero-octameric complex must contain these variants to increase T2D susceptibility. Our results support this notion because the enhanced GTP stimulatory effect afforded by the A1369 variant was not preserved in K_{ATP} channel subunits containing a combination of the E23, K23, S1369, and A1369 variants (Fig. 6).

From a physiological perspective, K_{ATP} channels encoded by the *KCNJ11* and *ABCC8* genes are expressed in several different tissues. The physiological consequence of the K23/A1369 T2D risk haplotype regarding enhanced MgATPase activity would be to suppress first-phase K_{ATP} channel–dependent insulin secretion. Although earlier studies on homozygous carriers of the K23 variant did not observe any reduction in insulin secretion (16,40), a recent clinical study demonstrated an impaired insulin secretory response in humans homozygous for the K23 variant (25). In addition, K_{ATP} channels are involved in the appropriate α-cell glucagon secretory response (41), and the K23/A1369 T2D risk haplotype may also deleteriously affect glucagon secretion. Subtle alterations in CNS K_{ATP} channel activity may also affect the regulation of whole-body glucose homeostasis via alterations in the excitability of hypothalamic neurons. Despite no overt clinical phenotype, subjects carrying two copies of the K23/A1369 T2D risk haplotype may be at an increased risk of developing diabetes in combination with other genetic variations or environmental factors, such as diet and lifestyle (42).

In summary, our results provide evidence that the *ABCC8* A1369 variant plays a major role in determining the properties of K_{ATP} channels containing the K23/A1369 T2D risk haplotype and describe a plausible molecular mechanism by which ~10% of the general population homozygous for this haplotype may be at increased risk for developing T2D.

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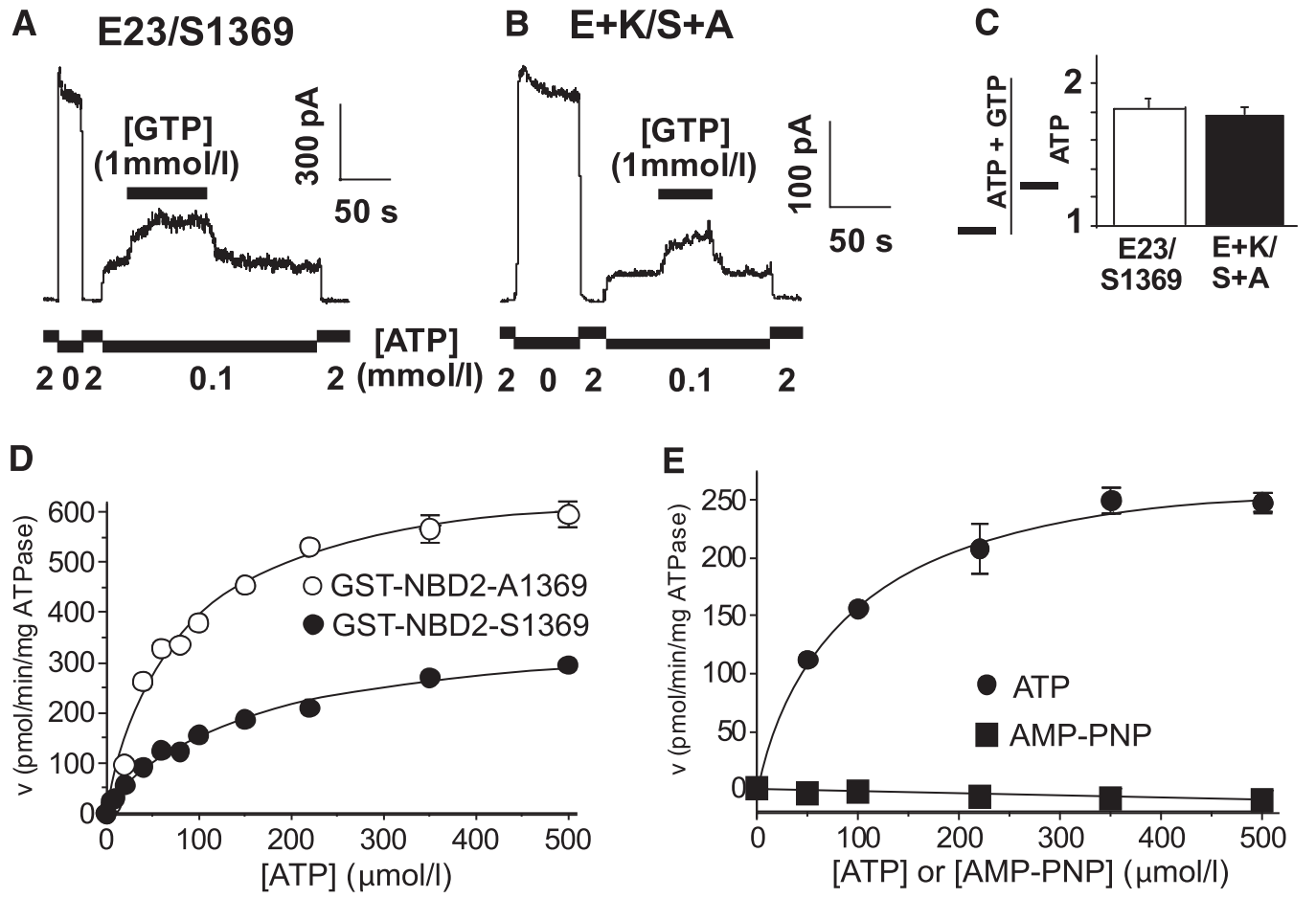

FIG. 7. The enhanced GTP stimulatory effect in K_{ATP} channels containing the *ABCC8* A1369 variant is lost on coexpression of the S1369 variant. *A*: Representative macroscopic current recordings of human K_{ATP} channel activity before and after exposure to 1 mmol/L GTP. K_{ATP} channels contained the E23 and S1369 variants. *B*: Representative macroscopic current recordings of human K_{ATP} channel activity before and after exposure to 1 mmol/L GTP. K_{ATP} channels contained the E23, K23, S1369, and A1369 variants (E+K/S+A) that represent the quasi-heterozygous channel composition. *C*: Grouped data (n = 19–25 patches per group) indicating no significant differences. *D*: Representative Michaelis-Menten plots of MgATPase activities from GST-NBD2 fusion proteins containing the S1369 or A1369 variant. *E*: The nonhydrolyzable ATP analog (AMP-PNP) abolishes the MgATPase activity of GST-NBD2 fusion protein containing the S1369 variant (■), compared with the activity in the presence of ATP (●).

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